Mating Disruption of Oriental Beetle (Coleoptera: Scarabaeidae) in Turfgrass Using Microencapsulated Formulations of Sex Pheromone Components

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ABSTRACT The feasibility of mating disruption in the oriental beetle, *Anomala orientalis* Waterhouse, with microencapsulated sprayable formulations of (Z)-7-tetradecen-2-one, the major sex pheromone component, was evaluated in turfgrass areas. The effect of the applications was measured by monitoring male *A. orientalis* captures in pheromone-baited traps throughout the flight period and estimating *A. orientalis* larval densities in September in soil/sod samples. Trap captures were 90–100% lower in the treated areas during the first 7–10 d after treatment, but started to increase thereafter. Therefore, applications were repeated after 14 d in most treatments. The formulation tested in 2002 and 2003 reduced trap captures by 87–88% with two applications of each 12.5 or 50 g pheromone/ha but only by 74% by a single application of 75 g pheromone/ha. Reductions of *A. orientalis* larval populations by 68–74% were not significant because of very high variability of larval densities in the nontreated areas. Two different formulations tested in 2004 were less effective. Significant amounts of the pheromone remained on grass foliage after application, but 51 and 73% of this residue were washed off the foliage with 3.2- and 6.4-mm post-treatment irrigation, respectively. Shoes walked at 1 day after treatment through pheromone-treated areas were sufficiently contaminated with pheromone to attract high numbers of *A. orientalis* males in nontreated areas. Mating disruption is a promising strategy for *A. orientalis* management in turfgrass. However, more persistent formulations need to be developed that have a lower potential to contaminate shoes and other clothing articles with pheromone.

KEY WORDS turfgrass, mating disruption, *Anomala orientalis*, sex pheromone, (Z)-7-tetradecen-2-one

IN THE NORTHEASTERN UNITED STATES, a complex of white grub species (Coleoptera: Scarabaeidae) is the most widespread and difficult to control of the turfgrass insect pests (Potter 1998, Vittum et al. 1999). Within this complex, the Japanese beetle, *Popillia japonica* Newman, has been considered the key pest species even though a significant amount of the damage attributed to this species is actually caused by the oriental beetle, *Anomala* (=*Exomala*) *orientalis* Waterhouse. *A. orientalis* has been erroneously considered a relatively minor pest until recently because adults are cryptic and largely go unnoticed, and the larvae of *P. japonica* and *A. orientalis* are indistinguishable without magnification. *A. orientalis* has become the most important turfgrass insect pest in New Jersey, southeastern New York, Connecticut, and Rhode Island (Alm et al. 1999, A.M.K., personal observations). In addition, it is also the major white grub species in ornamental nurseries and blueberries (S. Polavarapu 1996) and causes losses in cranberries, strawberries, raspberries, peaches, and sweet potatoes. The average composition of white grub populations in New Jersey turfgrass areas in fall 2001 and 2002 (130 sites including golf courses, athletic fields, home lawns) was 61% *A. orientalis*, 15% Asiatic garden beetle, *Maladera castanea* Arrow, 12% *P. japonica*, 8% northern masked chafer, *Cyclocephala borealis* Arrow, 2% European chafer, *Rhizotrogus majalis* (Razoumowsky), 2% May/June beetles (*Phyllophaga* spp.), and 2% green June beetle, *Cotinis nitida* L. (A.M.K., unpublished data). An increase in *A. orientalis* significance may occur in other areas where it is already established, i.e., all of coastal New England and Middle Atlantic states as well as Ohio, Virginia, North Carolina, South Carolina, West Virginia, and Tennessee (Potter 1998, Alm et al. 1999, Vittum et al. 1999).

Like all the above species, *A. orientalis* has a 1-yr life cycle. At the latitude of New Jersey, *A. orientalis* flight occurs from early June through early August, with peak flight activity typically in late June/early July.

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After mating, the females lay eggs among the roots of host plants, and the eggs hatch in 2–3 wk. The first and second instar each last around 3 wk so that by late August third instars start to appear, and by mid-September, the majority of the larvae are in the third instar (Potter 1998, Vittum et al. 1999, A.M.K., unpublished data). After overwintering below the frost line, the third instars resume feeding until pupation in late spring. The extensive feeding activity of the larger larvae can kill large areas of grass from mid-August into mid-October, especially under warm dry conditions (Potter 1998, Vittum et al. 1999). In addition, vertebrate predators can tear up the turf to feed on the grubs (Potter 1998, Vittum et al. 1999).

The sex pheromone of *A. orientalis* consists of a 9:1 blend of (Z)-7-tetradecen-2-one and (*E*)-7-tetradecen-2-one (Zhang et al. 1994, Facundo et al. 1994). Field trapping studies have indicated that, at concentrations >1 μg, (Z)-7-tetradecen-2-one alone is as effective as a 9:1 blend containing both compounds in attracting males (Facundo et al. 1994). Red rubber septa baited with (Z)-7-tetradecen-2-one in Trécé Japanese beetle are currently being used for monitoring adult *A. orientalis* populations (Alm et al. 1999). Intensive studies of mating and postmating behaviors and spatial and temporal distribution patterns of *A. orientalis* in turf and soil environments (Facundo 1997, Facundo et al. 1999a, b) suggested that sex pheromone–mediated mate acquisition and copulation occur at or near the soil surface, immediately after female emergence from the soil, close to the emergence site. Males respond to female-released pheromone by a combination of flying upwind and walking short distances (Facundo et al. 1999a). Both sexes are most active between 1800 and 2200 hours (Facundo 1999b).

Despite the proximity of turf to people, chemical insecticides are still the primary tools for white grub management. However, the implementation of the Food Quality Protection Act of 1996 (FQPA) (Anonymous 1996) has resulted in the loss of many insecticides for white grub control. The need for the development of safer effective alternatives is apparent. Mating disruption with sex pheromones is widely used as an environmentally safe, nontoxic alternative to broad-spectrum insecticides for several moth species (Cardé and Minks 1995). Even though the sex pheromones of scarab beetle have been studied intensively and are used for monitoring purposes, only recently has mating disruption technology been considered as a possibility for management of white grubs. In small scale experiments application of the major component of *A. orientalis*, sex pheromone resulted in 64–90% reduction in trap catches in cages treated with the pheromone compared with catches in untreated cages (Facundo 1997). Polavarapu et al. (2002) showed the feasibility of mating disruption in *A. orientalis* in large-scale field experiments in blueberries and ornamental nurseries with a microencapsulated sprayable formulation of its sex pheromone.

Because of the importance of *A. orientalis* as a turfgrass pest, we initiated a study to determine the feasibility of developing mating disruption technology for this pest also in the turfgrass environment using microencapsulated sprayable formulations. Unlike blueberry and ornamental nurseries, turfgrass areas are generally accessible and often meant to be used by many people. Therefore, we also wanted to determine whether contamination of clothing article such as shoes could be a potential problem by attracting male *A. orientalis* outside of treated areas.

Materials and Methods

Mating Disruption Field Trials

To determine the feasibility of mating disruption technology, we conducted field trials with a sprayable microencapsulated formulation of the *A. orientalis* sex pheromone developed using proprietary processes by 3M Canada Co. (London, Ontario, Canada) and Suterra (Bend, OR). The 3M formulation used in 2002 and 2003 contained 20% (Z)- and (*E*)-7-tetradecen-2-one at a 93:7 ratio. Because 3M discontinued the production of its formulation, two Suterra formulations were used in 2004 containing 5.35 (Suterra 03) and 24.11% (Suterra 04), respectively, of (Z)-7-tetradecen-2-one.

Assessment Methods

Two methods were used to determine the effect of treatments on the mating success of *A. orientalis.* The first method measured the ability of *A. orientalis* males to locate a pheromone source similar to a female by determining the number of *A. orientalis* males captured in traps. Trapping was also used to monitor *A. orientalis* male flight and optimize the application timing. The traps consisted of standard Japanese beetle traps (Trécé, Salinas, CA) baited with rubber septa lures containing (Z)-7-tetradecen-2-one and were fitted into a hole in the ground so that only the funnel portion remained above ground. The traps were placed in each plot at least 20 m from the plot's border and any other trap. In 2002, four traps with 300 μg (Z)-7-tetradecen-2-one per septum were placed per plot, and septa were replaced once after 4 wk. In 2003 and 2004, three traps with 30 μg (Z)-7-tetradecen-2-one per septum were placed per plot, and septa were replaced twice after 3 wk of use. Traps were first placed in early June of each year and emptied every 3–4 d and directly before treatment applications until beetle flight was very low. During the last 1 or 2 wk of the flight period, the traps were emptied only once per week. Captured males were counted and killed.

The second method estimated the densities of *A. orientalis* larvae during September after the applications. Larval densities were determined in the plots by taking 40 (2003) or 30 (2003 and 2004) soil/sod cores (10.8 cm diameter by 10 cm depth) with a standard golf hole cup cutter in a grid pattern at least 15 m inside from the plot's border. Any scarab larvae found in the cores were identified to species using the raster pattern.

Field Trial 2002. In 2002, field plots were situated at the Rutgers Research Station in Adelphia (NJ) (one replicate per treatment) and in the center area of the Monmouth Park Racetrack (MPR; Oceanport, NJ) (two replicates per treatment). The plots measured between 0.4 and 0.56 ha in size and were separated from other plots by a minimum of 100 m. The Adelphia plots at the Research Station were 3- and 4-yr-old tall fescue, *Festuca arundinacea* Schreb, fields mown at 3.8 cm. The plots at MPR were old turf stands composed of varying mixtures of tall fescue, fine fescues, *Festuca* spp., perennial ryegrass, *Lolium perenne* L., and Kentucky bluegrass, *Poa pratensis* L., and were mown at 7.6 cm. All plots were watered as needed to prevent excessive drought stress. To monitor *A. orientalis* male flight, traps were placed on 4 June 2002.

The treatment plots were broadcast sprayed twice with microencapsulated *A. orientalis* pheromone (3M formulation) at 50 g (AI)/ha. Applications were made on 11 June 2003 (Adelphia: 0700 hours, sunny, no wind, 18°C; MPR: 1500 hours, sunny, light wind, 29°C) and on 25 June 2003 (Adelphia: 0700 hours, sunny, no wind, 21°C; MPR: 0700 hours, sunny, no wind, 23°C) using a tractor-drawn boom sprayer in Adelphia (467-liter/ha spray volume, 207-kPa spray pressure) and a self-propelled boom sprayer unit in MPR (467 liter/ha, 276 kPa). The control plots were not sprayed. All plots received 6.4-mm overhead irrigation after treatment.

Field Trial 2003. In 2003, field plots were situated at the Rutgers Research Station in Adelphia (NJ), in the center area of the Monmouth Park Racetrack (MPR), and in irrigated roughs at the Spring Lake Golf Club (SLGC; Spring Lake, NJ). There was one replicate per treatment at each site. The plots measured between 0.42 and 0.55 ha in size and were separated from other plots by a minimum of 100 m. The plots at the Research Station were 3- and 4-yr-old stands of tall fescue (control), fine fescue (low application rate), and a mixture of Kentucky bluegrass and tall fescue (high application rate), all mown at 3.8 cm. At MPR, the plots were old turf stands composed of varying mixtures of tall fescue, fine fescues, perennial ryegrass, and Kentucky bluegrass, and were mown at 7.6 cm. At SLGC, the roughs were old stands composed of Kentucky bluegrass and perennial ryegrass and were mown at 7.6 cm. All plots were watered as needed to prevent excessive drought stress.

Two treatments were applied consisting of broadcast sprays with microencapsulated *A. orientalis* pheromone (3M formulation) at (1) 75 g (AI)/ha applied only on the first application date and (2) 12.5 g (AI)/ha applied on the first and second application date. Applications were made on 30 June 2003 (Adelphia: 0900 hours, sunny, no wind, 27°C; MPR: 1100 hours, sunny, light wind, 29°C; SLGC: 0800 hours, sunny, no wind, 26°C) and on 14 July 2003 (Adelphia: 0900 hours, sunny, no wind, 23°C; MPR: 0700 hours, sunny, no wind, 23°C; SLGC: 0900 hours, overcast, no wind, 23°C) using a tractor-drawn boom sprayer in Adelphia (467-liter/ha spray volume, 207 kPa) and a self-propelled boom sprayer unit at SLGC (934 liter/ha, 276 kPa). At MPR, treatments were applied with handgun units (934 liter/ha, 276 kPa) on the first date and with a self-propelled boom sprayer unit (467 liter/ha, 207 kPa) on the second date. The control plots were not sprayed. All plots received 6.4-mm overhead irrigation after treatment.

Field Trial 2004. In 2004, field plots were situated in irrigated roughs between tees and fairways at Shark River Golf Course (Neptune, NJ; one replicate + one extra control) and Hominy Hill Golf Course (Colts Neck, NJ; two replicates). The plots measured between 0.35 and 0.44 ha in size and were separated from other plots by a minimum of 130 m. All plots were old stands with various compositions of grass species but primarily Kentucky bluegrass and perennial ryegrass. All plots were watered as needed to prevent excessive drought stress. Traps were placed on 2 June 2004. Two treatments were applied consisting of broadcast sprays with microencapsulated *A. orientalis* pheromone in two different formulations, Suterra 03 and Suterra 04. Both formulations were applied at 25 g (AI)/ha on each of two application dates. Applications were made on 12 June 2004 (Shark River GC: 0700 hours, sunny, no wind, 20°C; Hominy Hill GC: 0700 hours, sunny, no wind, 25°C) and 24 June 2004 (Shark River GC: 0700 hours, sunny, no wind, 22°C; Hominy Hill GC: 1900 hours, sunny, no wind, 27°C) using self-propelled boom sprayer units (934 liter/ha, 276 kPa). The control plots were not sprayed. All plots received 6.4-mm overhead irrigation after treatment.

Effect of Postapplication Irrigation on Pheromone Adherence to Grass Blades

Removal of grass clippings from mowing is a common practice on fairways of many golf courses. After spray application, a significant amount *A. orientalis* pheromone may remain on the grass foliage rather than drip off into the thatch and soil. This pheromone proportion would than be removed with the clippings, thus reducing the efficacy of the pheromone application. An experiment was conducted to determine how much pheromone would remain on the foliage and whether postapplication irrigation would wash the pheromone into the thatch and upper soil layers.

In a Kentucky bluegrass area mown at 3.8 cm on the Rutgers University Horticultural Farm 2 (North Brunswick, NJ), 1-m^2 turf plots were sprayed (backpack sprayer, 221 kPa, 1,000 liter/ha) with *A. orientalis* sex pheromone (3M formulation) at a rate of 75 g (AI)/ha followed by 0, 3.2, and 6.4 mm of postapplication irrigation using a watering can. Control plots were neither sprayed nor irrigated. The plots were distributed in a randomized complete block design with three replicates per treatment. There was a spacing of 4 m between replicates. After the grass was allowed to dry, sod/soil cores (5.1 cm diameter by 5.1 cm) were taken with precut 10.2-cm-length PVC pipe sections. The cores were pushed through the section so that the thatch surface was level with the section's edge, the grass was cut just above the thatch surface, and the clippings were collected in a 250-ml wide-

mouth glass jar. From each replicate, six cores were taken, and the clippings were pooled by replicate. The jar was sealed and brought to the laboratory for pheromone extraction. One hundred milliliters of acetone was added per jar, and the jar contents were stirred, allowed to sit for 1 min, and washed into a 125-ml glass bottle through a strainer to remove the grass clippings. The bottles were shipped in a cooler to the USDA-ARS Chemicals Affecting Insect Behavior Laboratory (CAIBL; Beltsville, MD). The samples were purified and concentrated before analysis. The acetone from each sample was evaporated by a rotary evaporator, and the residue was extracted with 24 ml (3 by 8 ml) of hexane. The concentrated sample was further purified by column chromatography using 20 g of silica gel and 80 ml of ethyl acetate in hexane (5:100). The elution was condensed to 1 ml through rotary evaporation. The samples were analyzed by gas chromatography–mass spectrometry (GC-MS) (Hewlett-Packard 6890 GC coupled to a Hewlett-Packard 5973 Mass Selective Detector using a 60 m by 0.25-mm ID, 0.25-μm film thickness DB-WAXETR capillary column (J&W Scientific, Folsom, CA) at 50°C for 2 min, ramped at 15°C/min to 230°C, and held at this temperature for 15 min). Synthetic pheromone component, (Z)-7-tetradecen-2-one, was used as the standard for quantitative determination. A second set of samples was taken at 7 days after treatment and analyzed using the same methodology.

Adsorption of Oriental Beetle Sex Pheromone to Shoes

A. orientalis pheromone can adsorb to surfaces it comes into contact with. These can attract male *A. orientalis* over an extended period of time depending on the degree of contamination and the type of material. This could be a nuisance to people that come into contact with pheromone-treated turfgrass. On golf courses, shoes would be the most likely clothing item to be contaminated. To test whether shoes can be contaminated with enough *A. orientalis* pheromone to cause potential nuisance, athletic shoes (Athletic Works Silverseries Gray, size 10W) were walked for 30 min through the same areas used for the mating disruption field trials after treatments had been applied. The shoes were placed by pair in shoe boxes in a cooler, brought to the laboratory, and placed at −18°C to stop pheromone volatilization. From each pair, one shoe was used for pheromone extraction and the other in a bioassay.

The shoes used for the bioassay were thawed and lined up on the surface of a nonpheromone-treated Kentucky bluegrass area on the Rutgers University Horticultural Farm 2 (East Brunswick, NJ). Shoes were lined up perpendicular to the prevailing wind direction in a continuous line of three groups, with each group containing one shoe from each treatment and a non–pheromone-exposed shoe. The distance between shoes was 4.5 m. The position of shoes in each group was random and was changed in each repetition of the experiment. In the 2004 bioassay, one Trécé Japanese beetle trap baited with rubber septa containing 30 μg *A. orientalis* sex pheromone was placed at each end of the line, 6 m away form the last shoe, as a standard. *A. orientalis* males attracted to the traps or the shoes and soil surface within 5 cm of the shoes were destructively collected after 15, 30, and 45 min. In 2003, shoes were walked through each of the replicates of the mating disruption trial that had been treated with 75 g (AI)/ha on 30 June 2003. One pair of shoes was walked in each of the replicates at 1 DAT and another pair at 8 DAT. The bioassay was conducted at 13 DAT and 19 DAT. In 2004, one pair of shoes per replicate was walked at 1 DAT through each replicate of both pheromone formulations (both treated with 25 g [AI]/ha on 12 June 2004), and three bioassays were conducted on 3 consecutive d starting on 24 June 2004. In between bioassays, the shoes were kept at −18°C. Careful attention was given to keep the shoes separated from each other to prevent contamination.

To extract *A. orientalis* pheromone, shoes were thawed at room temperature and held above a glass pan (33 cm by 23 cm), and soles and sides (up to 5 cm from bottom of sole) were rinsed with a solvent using a wash bottle. The rinsate was collected in 500-ml glass bottles that were shipped in a cooler to the USDA-ARS CAIBL for analysis. In 2003, each shoe was rinsed with 500 ml acetone and left upright in the glass pan for another 10 min. The samples and control were purified and analyzed using the same methodology as described for the grass blade extracts. Because acetone samples contained too much undesired organic compounds that interfered with analysis of pheromone component, in 2004, each shoe was rinsed with 200 ml hexane and left upright in the glass pan for another 5 min. The samples and control were analyzed by GC-MS without concentration and purification using the select ion monitoring (SIM) method (Shimadzu OP5050A GC-MS equipped with a 30 m by 0.25 mm ID, 0.25-μm film thickness DB-5 capillary column; J&W Scientific) at 100°C for 2 min, ramped at 15°C/min to 280°C, and held at that temperature for 15 min, using m/z 82 and 125 as the monitoring ions). Synthetic pheromone component, (Z)-7-tetradecen-2-one, was used as the standard for quantitative determination.

Statistics

Data were normalized where necessary by log (x + 1) transformation (number of males trapped before and after the first application in mating disruption trial, amount of pheromone extracted from shoes and grass clippings) or square-root transformation (number of *A. orientalis* larvae found per plot in May and in September in the mating disruption trial, number of males collected from the shoes). The data were analyzed using analysis of variance (ANOVA) with sampling or bioassay date as co-factors where appropriate and means separated with Tukey's test. Differences among means (±SE) were considered significant at $P < 0.05$.

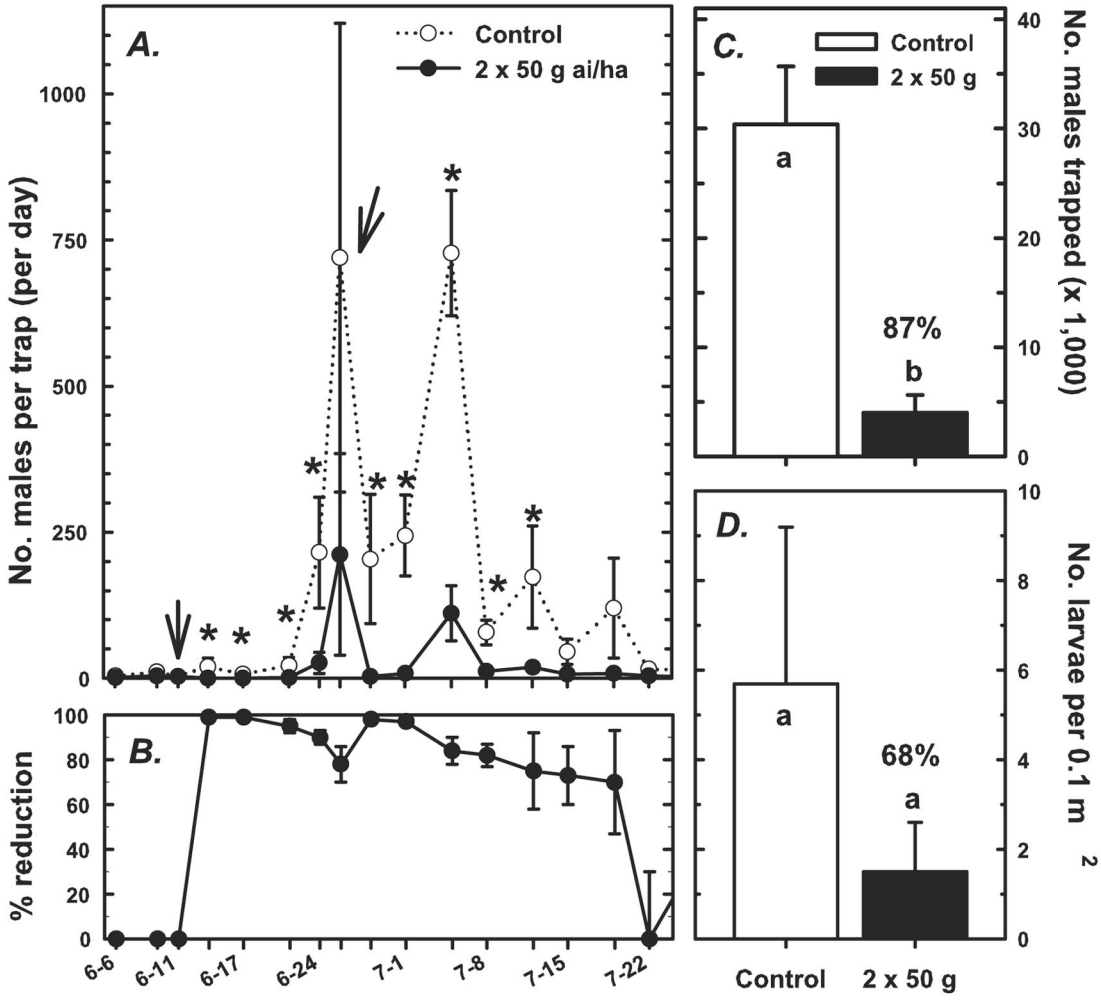

Fig. 1. Field season 2002 *A. orientalis* mating disruption trial. (A) Twice-weekly male trap captures (arrows indicate application dates). (B) Percentage reduction in twice-weekly trap captures. (C) Total seasonal trap captures. (D) *A. orientalis* larval densities in September after application. (A) Asterisk above data points indicates significant difference among means on sampling date. (C and D) Means with same letter above bars are not significantly different, and figures above bars indicate percent reduction compared with control.

Results

Mating Disruption Field Trials

Field Season 2002. The number of *A. orientalis* larvae recovered from samples in the plots during May 2002 before the application did not differ significantly among plots designated for the different treatments (range, 5.1–11.8/0.1 m^2). *A. orientalis* male flight started in the first week of June, and trap captures had two distinct peaks on 25 June 2002 and around 5 July 2002 (Fig. 1A). Total trap captures before pheromone application did not differ significantly between control plots (85.2 ± 25.8) and treatment plots (233.3 ± 185.7). Trap captures after the first pheromone application were significantly affected by treatment (F = 113.18; df = 1,64; $P < 0.001$) and day after application (F = 20.5; df = 15,64; $P < 0.001$), with no significant interaction between treatment and day after application (P = 0.09). Trap captures were 87% lower in the treated plots (4,002.3 ± 1,604.5) than in the control plots (30,391.7 ± 5,285.1; Fig. 1C). During the 62-d trapping period after treatment application, trap captures were significantly lower in the treated plots than in the controls on days 2–13 and days 17–27 after treatment ($F \geq 8.9$; df = 1,4; $P < 0.05$) but not on day 14 and days 31–62 after treatment, indicating that the effect of the pheromone only lasted ≈2 wk after each application. Accordingly, percent reduction in trap captures (Fig. 1B) in the treated plots was 96–100% for the first week after each application but started to drop during the second week.

A. orientalis larval densities in September could only be compared within the MPR plots because the control plot in Adelphia had mistakenly been treated with

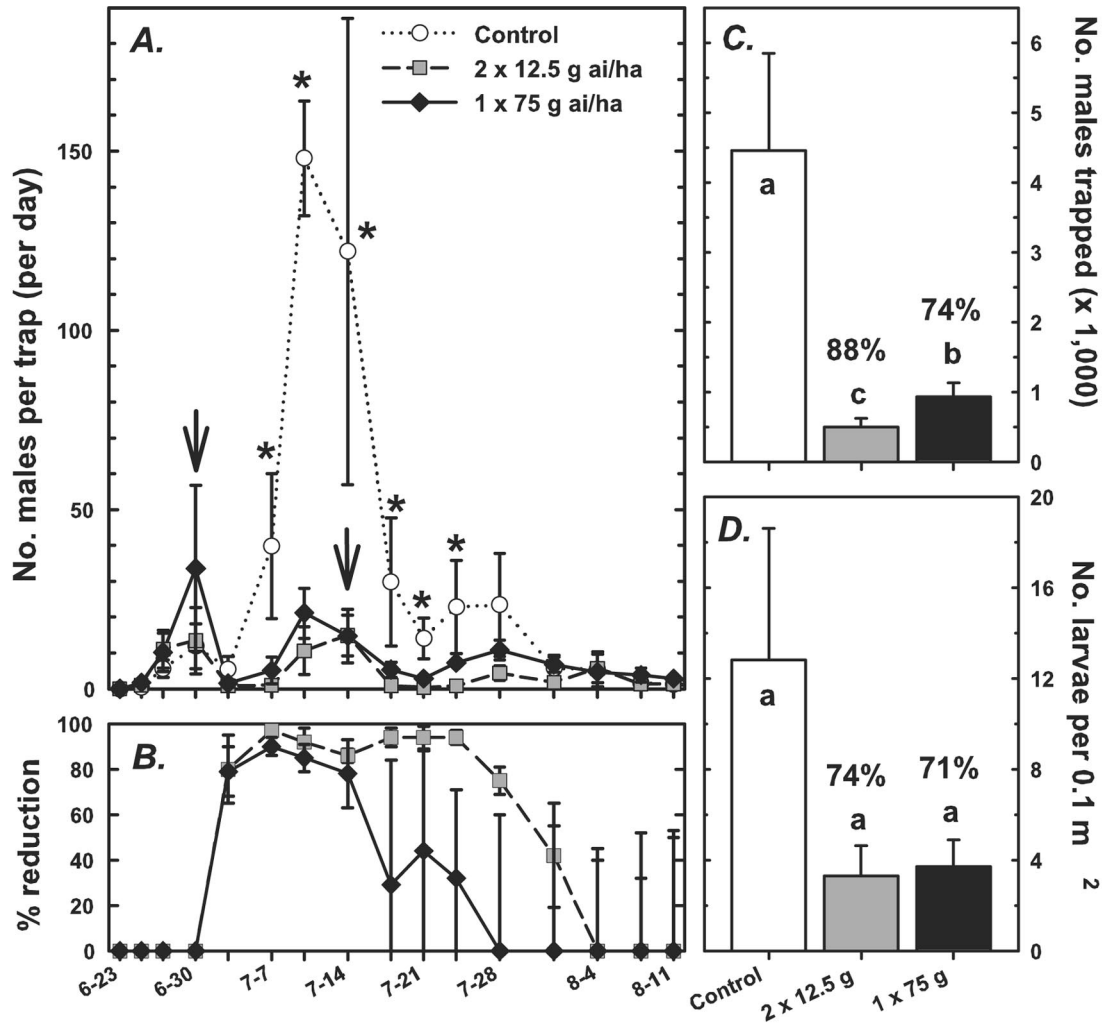

Fig. 2. Field season 2003 *A. orientalis* mating disruption trial. (A) Twice-weekly male trap captures (arrows indicate application dates). (B) Percentage reduction in twice-weekly trap captures. (C) Total seasonal trap captures. (D) *A. orientalis* larval densities in September after application. (A) Asterisk above data points indicates significant difference among means on sampling date. (C and D) Means with same letter above bars are not significantly different, and figures above bars indicate percent reduction compared with control.

the insecticide imidacloprid in July. In all fields, larval populations consisted of >95% *A. orientalis*, the remainder being *P. japonica*, *C. borealis*, and *M. castanea.* At MPR, one of the control plots had received less irrigation than the other three plots. There were only two *A. orientalis* larvae per 0.1 m^2 compared with 9.5 per m^2 in the other control plot. Larval densities in the treated plots were 68% lower than in the treated plots, but the reduction was not statistically significant because of the high variation and low number of replicates (Fig. 1D). At Adelphia, the treated plot had 10.4 *A. orientalis* larvae per 0.1 m^2, whereas no larvae were found in the Merit-treated control plot, which had 4.8 times higher trap captures than the treated plot.

Field Season 2003. The number of *A. orientalis* larvae recovered from samples in the plots during May 2003 before the application did not differ significantly among plots designated for the different treatments (range, 5.6–10.2/0.1 m^2). *A. orientalis* male flight started in the last week of June, and trap captures had a distinct peak around 10–14 July 2003 and continued elevated activity until 28 July 2003 (Fig. 2A). Total trap captures before pheromone application did not differ significantly between the plots assigned to the control (144.5 ± 70.8), the plots to be treated with 2 × 12.5 g (AI)/ha (194.5 ± 97.8), and the plots to be treated with 1 × 75 g (AI)/ha (374.4 ± 224.1).

Trap captures after pheromone application were significantly affected by treatment ($F = 30.14$; df = 2,90; $P < 0.001$) and day after application ($F = 17.71$; df = 14,90; $P < 0.001$), with a significant interaction between treatment and day after application ($F = 2.86$; df = 28,90; $P < 0.001$; Fig. 1C). Total trap captures were significantly lower in the 1 × 75 g (AI)/ha

treatment (926.2 ± 204.2; 74% reduction) than in the control (4,452.1 ± 1,395), and significantly lower in the 2 × 12.5 g (AI)/ha treatment (497.4 ± 126.3; 88% reduction) than in the 1 × 75 g (AI)/ha treatment. During the 56-d trapping period after the first application, trap captures compared with the control were significantly lower ($F \geq 5.79$; df = 2,6; $P < 0.05$) on days 7–24 in the 2 × 12.5 g (AI)/ha treatment, but only on days 7 and 14 in the 1 × 75 g (AI)/ha treatment; trap captures never differed significantly between the two treatments. Percent reduction in trap captures (Fig. 1B) was only 79–80% for both treatments at 4 d after the first application and stayed at 94–97% between 7 and 24 d after the first application in the 2 × 12.5 g (AI)/ha treatment before dropping quickly, and only at 78–90% between 7 and 14 d after the first and only application in the 1 × 75 g (AI)/ha treatment before quickly dropping.

White grub populations in the plots in September 2003 consisted of 91% *A. orientalis*, 5% *P. japonica*, 2% *M. castanea*, and 2% *C. borealis*. *A. orientalis* larval densities did not differ significantly among treatments ($F = 3.55$; df = 2,6; $P = 0.09$) because of the high variability particularly among the control plots (range, 6.3–24.3/0.1 m^2), with 12.8 ± 5.8 larvae per 0.1 m^2 in the control, 3.3 ± 1.4 larvae per m^2 in the 2 × 12.5 g (AI)/ha treatment (74% reduction), and 3.6 ± 1.2 larvae per 0.1 m^2 in the 1 × 75 g (AI)/ha treatment (71% reduction; Fig. 1D).

Field Season 2004. The number of *A. orientalis* larvae recovered from samples in the plots during May 2004 before application did not differ significantly among plots designated for the different treatments (range, 1.9–3.1/0.1 m^2). *A. orientalis* flight started in the first week of June, had an extended peak between 17 June and 5 July 2004, and continued elevated activity until ≈20 July 2004 (Fig. 3A). Total trap captures before pheromone application did not differ significantly between the plots assigned to the different treatments and were 335.1 ± 112.3 in the control plots, 266.9 ± 76.2 in the plots to be treated with the Suterra 03 formulation, and 226.4 ± 68.8 in the plots to be treated with the Suterra 04 formulation.

Trap captures after pheromone application were significantly affected by treatment ($F = 52.74$; df = 2,98; $P < 0.001$) and day after application ($F = 14.54$; df = 13,98; $P < 0.001$), with a significant interaction between treatment and day after application ($F = 3.23$; df = 26,98; $P < 0.001$). Total trap captures were significantly lower for the Suterra 03 (1,474.8 ± 312.9; 68% reduction) and Suterra 04 formulations (1448.3 ± 318.3; 70% reduction; both applied at 2 × 25 g (AI)/ha) than in the control (4,650.8 ± 959.2; Fig. 3C). During the 51-d trapping period after treatment application, trap captures compared with the control were significantly lower ($F \geq 5.37$; df = 2,7; $P < 0.05$) on days 1–16 and day 23 in the Suterra 04 treatment and on days 1–9 and days 16–23 in the Suterra 03 treatment; trap captures never differed significantly between the two treatments. Percent reduction in *A. orientalis* male captures (Fig. 1B) in the treated plots after the first application was >90% for both formulation during the first week but declined to 39% after 2 wk for Suterra 03 and to 65% for Suterra 04. After the second application, the reduction in trap captures compared with the untreated plots declined somewhat slower for Suterra 03 than for Suterra 04 but were <50% for both 2 wk after application.

White grub populations in the plots in September 2004 consisted of 64% *A. orientalis*, 20% *P. japonica*, 6% *C. borealis*, 5% *M. castanea*, and 5% *Phyllophaga* sp. *A. orientalis* larval densities in September were 6.0 ± 1.7 larvae per 0.1 m^2 in the control plots, 7.3 ± 3.4 larvae per 0.1 m^2 in the plots treated with Suterra 03 (22% higher then control), and 2.7 ± 0.7 larvae per 0.1 m^2 in the plots treated with Suterra 04 (56% reduction). Because of the high variation in larval densities, there were no significant differences among treatments.

Effect of Postapplication Irrigation on Pheromone Adherence to Grass Blades

No pheromone was detected in the control samples on both sampling dates and this treatment was not included in the analysis. In the samples taken directly after application, significantly ($F = 7.48$; df = 2,6; $P = 0.02$) less pheromone was detected in clippings taken from plots watered with 3.2 (3.6 ± 0.7 μg) and 6.4 mm (2.7 ± 0.3 μg) than in the nonwatered plots (7.3 ± 1.3 μg). In the samples taken at 7 DAT, no pheromone could be detected in all samples except one from the non–watered-in replicates (0.002 μg).

Adsorption of Sex Pheromone to Shoes

In the 2003 experiments (shoes walked for 30 min through the replicates treated with the 3M formulation at 75 g [AI]/ha), GC-MS determined the amount of pheromone rinsed off the shoes to be 62.1 ± 15.3 μg per shoe on shoes walked 1 DAT. No pheromone was detected on the shoes walked 8 DAT and on the control shoes. In the bioassay, no males were attracted to the control shoes, and these data were not included in the analysis. *A. orientalis* male attraction was significantly affected by treatment ($F = 51.65$; df = 1,8; $P < 0.001$) and bioassay date ($F = 13.29$; df = 1,8; $P < 0.01$), but there was no interaction between treatment and bioassay date. Significantly fewer males were attracted to shoes walked at 8 DAT (mean, 1.8 ± 1.6; range, 0–10) than to shoes walked at 1 DAT (mean, 42.3 ± 12.1; range, 6–81).

In the 2004 experiments (shoes walked at 1 DAT for 30 min through the replicates treated with the Suterra 03 or Suterra 04 formulation at 25 g [AI]/ha), GC-MS determined the amount of pheromone rinsed off the shoes to 36.1 ± 6.4 μg per shoe for Suterra 03 formulation, 15.7 ± 7.9 μg for the Suterra 04 formulation, and no pheromone could be detected in the control. Mean pheromone per shoe did not differ significantly between the two formulations. In the bioassay, no *A. orientalis* males were attracted to control shoes, and these data were not included in the analysis. Because the number of *A. orientalis* males coming to the traps varied considerably between bioassay dates (mean,

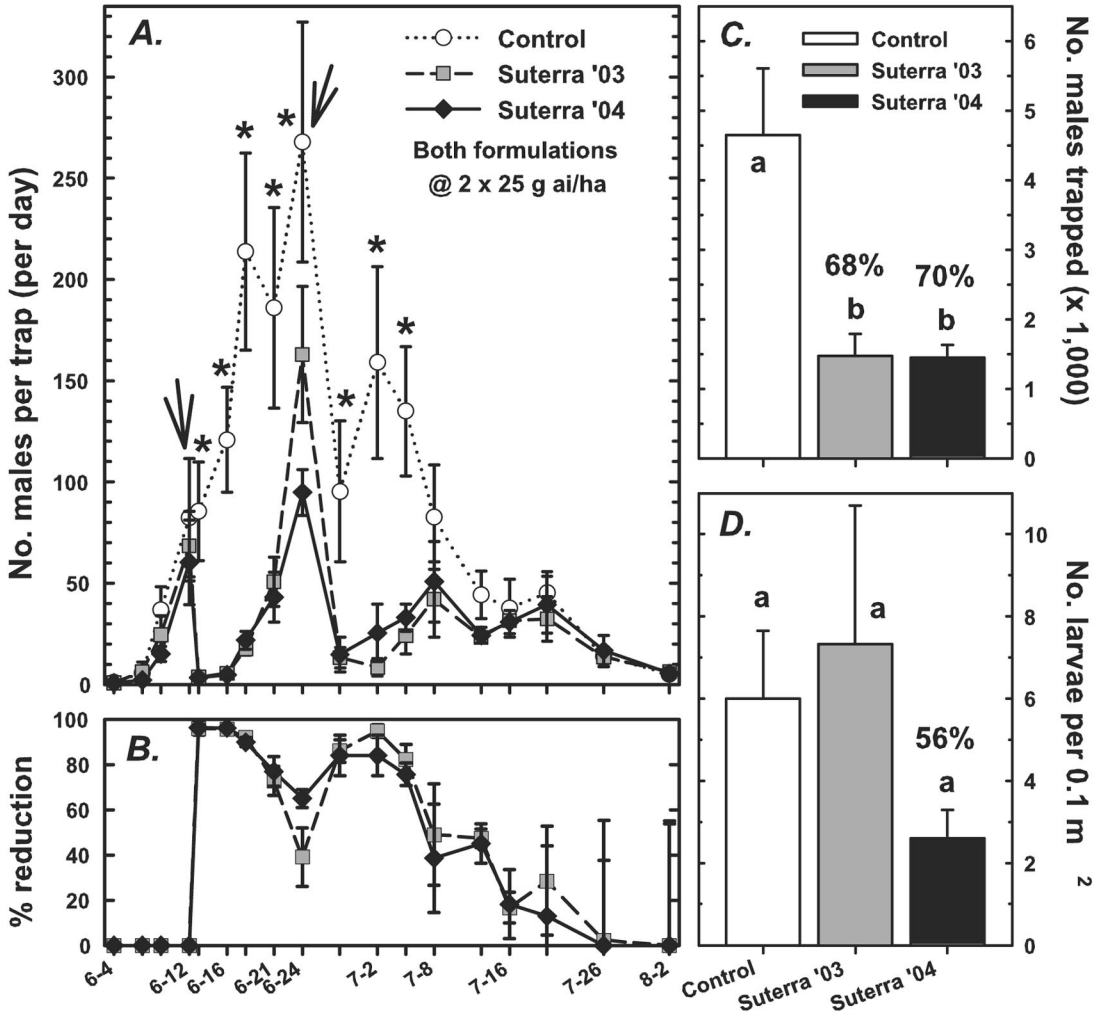

Fig. 3. Field season 2004 *A. orientalis* mating disruption trial. (A) Twice-weekly male trap captures (arrows indicate application dates). (B) Percentage reduction in twice-weekly trap captures. (C) Total seasonal trap captures. (D) *A. orientalis* larval densities in September after application. (A) Asterisk above data points indicates significant difference among means on sampling date. (C and D) Means with same letter above bars are not significantly different, and figures above bars indicate percent reduction compared with control.

101–170), the data were standardized by multiplying each data point with the average trap capture over all three bioassay dates divided by the average trap capture of the data point's bioassay date. There was no significant effect of bioassay date and no interaction between day and formulation. The average number of *A. orientalis* males per shoe (overall range, 6–101) did not differ significantly between the Suterra 03 formulation (34.2 ± 9.0) and the Suterra 04 formulation (21.4 ± 3.5; ($F = 1.93$; df = 1,12; $P = 0.19$).

Discussion

A previous study by Polavarapu et al. (2002) had shown the feasibility of using a microencapsulated formulation of sex pheromone components to disrupt sexual communication in *A. orientalis* in highbush blueberries and ornamental nurseries. This study showed that this technology is also feasible in the turfgrass system. However, the duration of the effect differed among systems. Using the same pheromone formulation (developed by 3M), a single spray at the beginning of the beetle's flight period reduced total seasonal trap captures by 92–97% in blueberries, 82–92% in ornamental nurseries, but only 74% in turfgrass. In turfgrass, the effect of the pheromone spray started to wane after ≈10 d, making a second application after 14 d necessary to obtain 87–88% reductions in seasonal trap captures. The two Suterra formulations used in the 2004 turfgrass experiment appeared to persist shorter then the 3M formulation and resulted in only 68–70% reductions in trap capture despite two applications.

Polavarapu et al. (2002) did not evaluate the effect of the pheromone sprays on *A. orientalis* larval populations because obtaining useful grub counts was too expensive and destructive in the blueberries and ornamental nurseries systems. In contrast, the turfgrass system allowed evaluating the effect on larval *A. orientalis* populations. However, because of the inherently high variability of white grub populations within and among turfgrass sites, the larval counts in our experiments, particularly in the nontreated areas, were too variable to allow for the detection of statistically significant difference in any of the three field seasons. Nevertheless, the trend in the first two field seasons (2002 and 2003) using the 3M formulation was very consistent, with 68–74% lower *A. orientalis* larval populations in the treated areas. In contrast, the Suterra 04 formulation reduced larval densities by only 56%, and the Suterra 03 formulation did not reduce larval densities.

Given the limited persistence of the pheromone sprays in the turfgrass system, irrespective of pheromone application rate, we believe that the efficacy of mating disruption using sprayable formulations could be improved with more frequent applications. For example, three applications at 10-d intervals would cover the critical parts of the flight period, with reductions in trap captures remaining >90%, probably even with lower pheromone application rates than used in this study, i.e., <12.5 g (AI)/ha per application. However, the availability in turfgrass of insecticides that are highly effective and only requiring one seasonal application (i.e., imidacloprid, clothianidin) will limit the acceptance of mating disruption unless a formulation can be developed that is more effective and/or requires only one seasonal application. We don't believe that this goal can be achieved using microencapsulated sprayable formulations.

In addition, the potential contamination of shoes and other clothing articles by the sprayable formulation and the ensuing attraction of male beetles to these articles outside of treated areas present a liability to these formulations. While no complaints were reported to us by golfers from the three golf courses on which our experiments were conducted, our bioassays and own experiences clearly show that this potential nuisance exists. Our field experiment on the effect of post-treatment irrigation clearly showed that larger amounts of pheromone remained on the grass foliage if no post-treatment irrigation was applied. This shows that the contamination potential would be even greater if the treated areas did not receive post-treatment irrigation as may be the case on golf roughs or in many landscape turf situations.

Sciarappa et al. (2005) showed that *A. orientalis* mating disruption could be also achieved with retrievable pheromone sources. As few as 50 pheromone sources per hectare each containing 1 (ChemTica dispensers) or 0.1 g (red rubber septa) placed at the beginning of the flight period in highbush blueberry fields reduced male trap captures by 95–99% and grub densities in sentinel potted blueberry bushes by 85–100%. The typical use pattern of many turf areas and the need for regular mowing make this approach impractical in turfgrass. However, we believe that dispersible pheromone formulations consisting of numerous broadcast small pheromone sources or fewer larger sources similar to the retrievable sources may solve the problems of limited persistence and contamination of clothing articles in the turfgrass system. Such formulations could make mating disruption an effective, safe, environmentally, and economically sound, easily implementable, durable, and highly integrated pest management–compatible option for *A. orientalis* management in turfgrass. Our future efforts will therefore concentrate on the development of dispersible formulations.

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